



How to Cite:

Ndawakai, N., Théophile, B., Pierre, S., & Elias, N. N. (2025). Larvicidal and adulticidal effects of essential oils of *Corymbia citriodora* (Myrtaceae) and *Xylopi aetiopica* (Annonaceae) on *Anopheles gambiae sensu stricto* Giles 1902. *International Journal of Health Sciences*, 9(S1), 258–271.

<https://doi.org/10.53730/ijhs.v9nS1.15640>

Larvicidal and adulticidal effects of essential oils of *Corymbia citriodora* (Myrtaceae) and *Xylopi aetiopica* (Annonaceae) on *Anopheles gambiae sensu stricto* Giles 1902

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Abstract---With the objective of combating malaria by reducing the populations of *Plasmodium* spp vectors, the larvicidal and adulticidal effects of essential oils of *Corymbia citriodora* (Myrtaceae) and *Xylopi aetiopica* (Annonaceae) on *Anopheles gambiae sensu stricto* Giles 1902 were determined. Biological tests were carried out using a methodology inspired by the standard WHO protocol (2005) and

revealed that the essential oils tested on larvae and adults of *Anopheles gambiae* sensu stricto Giles 1902 possess remarkable larvicidal and adulticidal properties. In stage II larvae, LC₅₀ values (ppm) were 27.07 and 42.88 respectively for *Corymbia citriodora* and *Xylopi aetiopica* with HL₅₀s of 1h 09min 32sec and 2h 41min 02sec respectively. In stage IV larvae, LC₅₀ values (ppm) were 30.62 (*Corymbia citriodora*) and 47.85 (*Xylopi aetiopica*) with HL₅₀s of 1h 40min 25sec and 3h 40min 41sec respectively. Adult LC₅₀s (ppm) for *Corymbia citriodora* and *Xylopi aetiopica* were 32.25 and 35.13 respectively. Adult HL₅₀s were 1h 50min 24sec and 2h 11min 46sec. *Corymbia citriodora* plant oil, which showed correspondingly low LC₅₀s and HL₅₀s, is therefore the most effective compared with *Xylopi aetiopica* plant oil.

Keywords---*Anopheles gambiae* ss, Essential oils, Local plants, Malaria.

1. Introduction

Mosquitoes are involved in the transmission of several pathogens responsible for diseases such as chikungunya, filariasis, encephalitis, Rift Valley fever, yellow fever, Zika fever and malaria (Mouchet *et al.*, 2004). Malaria is a parasitic disease caused by a hematophagous protozoan of the genus *Plasmodium*, transmitted to humans by the bite of an infested female mosquito of the genus *Anopheles* (Carnevale *et al.*, 2009). This infectious disease continues to affect mainly pregnant women and children under the age of five (Minsanté, 2009). Worldwide, the population likely to be infected by the parasite is expected to reach 247 million by 2021, with 619,000 deaths recorded (OMS, 2022). Unfortunately, Africa remains the continent most affected, with 234 million cases recorded, i.e. 95% (OMS, 2022). In Cameroon, the number of malaria cases recorded in health centers is 6,291,256, with 11,233 deaths (OMS, 2020). The three northern regions (Far North, North and Adamaoua) and the East are the hardest hit, with mortality rates of 32%, 27%, 29% and 19% respectively (PNLP, 2019). Faced with this situation, the country has made intermittent preventive treatment (IPT) for children under five and pregnant women its top priority. It has also opted for mass awareness-raising through the Long-Lasting Insecticide-Treated Mosquito Net distribution campaign. Unfortunately, the use of these products has proved harmful to animals, humans and the environment, due to their high toxicity and non-biodegradable state (Chandre *et al.*, 2000; Djogbénu, 2009). In addition to these harmful effects, vectors are developing resistance to insecticides (Carnevale *et al.*, 2009). Faced with these obstacles, the World Health Organization is proposing new strategies based on the use of plant products, which appear to be highly effective, available and biodegradable, thus encouraging innovation and the search for alternative solutions that would reduce production costs (OMS, 2020). In view of the limitations of synthetic insecticides and the increasing likelihood of using natural insecticides to control malaria vectors, we have chosen two local plants for their known insecticidal action: *Corymbia citriodora* (Myrtaceae) and *Xylopi aetiopica* (Annonaceae). The general objective of this work is to contribute to the fight against malaria by reducing the populations of *plasmodium* vectors.

2. Materials And Methods

2.1. Collection and identification of collected plants

The plants used in this work and identified at the Yaoundé National Herbarium under the numbers 4046 SRFK for *Corymbia citriodora* and 25091SRFK for *X. aetiopica* were collected in August 2020, when development and floristic diversity are at their peak. These samples were collected in the afternoon from 4pm to 6pm. This time slot was chosen to coincide with the time of day when people collect these plants to combat mosquitoes.

2.2. Drying of harvested plant parts

The harvested plant parts (Table I) were first cleared of debris and then dried in the open air and out of the sun for 07 days. These dried parts were then removed, crushed using a traditional mortar and sieved using a 0.1mm mesh sieve to obtain very fine powders.

Table I: Plant parts harvested

Plants	Part harvested
<i>Corymbia citriodora</i>	Leaves
<i>Xylopi aetiopica</i>	Fruits

2.3 Extraction of essential oils

Essential oils from the two plant essences were extracted by hydrodistillation using Clevenger equipment. A quantity of powder from each plant was hydrodistilled for 2 hours in the Dean Spark apparatus. The quantity hydrodistilled depended on the capacity of the flask (200 mL), and the operation was repeated five times to obtain a significant quantity of essential oil. 500g of powder from each plant was used for extraction. The oil, being less dense than the supernatant water, was collected from the Clevenger tap, then filtered with anhydrous sodium sulfate, and kept cold at -4°C until bioassay testing. From the total volume of oil obtained, the yield for each plant was calculated according to the formula below.

$$\text{Extraction Yield} = \frac{\text{Weight of essential oil}}{\text{Weight of plant material used}} \times 100$$

2.4. Determination of concentration ranges for larvicidal and adulticidal tests

Concentration ranges were selected on the basis of the WHO (2005) standard protocol (OMS, 2005) for assessing the sensitivity of mosquito larvae and adults to insecticides. The WHO recommends using a discriminating concentration, multiplied by 5 or 10 for known insecticides (Deltamethrin 0.05; i.e. 55 ppm). Thus, after three repetitions, the following concentration ranges (Table II) were selected.

Table II: Concentration ranges selected

Tests	Concentration ranges (ppm)				
Larvicidal	25	35	50	75	100
Adulticidal	30	40	55	100	150

2.5. Determining the larvicidal and adulticidal effect of essential plant oils

2.5.1. Larvicidal tests

The tests consisted in assessing the mortality of *Anopheles gambiae* ss larvae in the presence of diluted solutions of the extracts, following a methodology inspired by the WHO (2005) protocol. For this purpose, 100 batches of 25 larvae, i.e. 50 batches for stage II larvae and 50 batches for stage IV larvae, all hatching from soaked eggs from the Organization for the Coordination of Endemic Diseases in Central Africa (OCEAC), were collected using a bulb pipette and placed in 100 small transparent plastic dishes measuring 10 x 6 x 3.6 cm, each containing one volume of the stock solution (diluted solution of essential oil) supplemented with one volume of water up to 100 mL, total volume. Dead larvae were counted in hourly increments. First every 30 minutes, then every 1 hour, then every 2 hours for 12 hours of exposure to the different extract concentrations, and the device was maintained until 24 hours, at which time mortality was assessed. A larva is considered dead when it remains immobilized at the bottom of the dish and does not respond to touch with a needle.

2.5.2. Adulticidal tests

Adulticidal activity was assessed according to standard WHO protocol in CDC (Centers for Disease Control and Prevention) bioassay bottles, to evaluate the sensitivity of imaginal populations to different essential oils. The CDC bottles were thoroughly washed, dried and numbered from 1 to 5. Each number carries the concentration indicated. Each concentration was picked up with a propette and introduced into the bottle. The bottle was then shaken regularly to homogenize the interior, and placed in vertical positions to dry. After drying, twenty (20) unsexed 4-day-old adult mosquitoes were collected using a mouth aspirator and then introduced into each dried bottle corresponding to each concentration. Once the first mosquitoes were in the bottle, a stopwatch was started and observation began. The cumulative count of dead adults of *Anopheles gambiae* ss was made by time slot. First, after every 30 min, then at the 6th hour, then at the 12th and 18th hour of exposure. The system was finally abandoned to carry out the last observation after 24 hours of exposure.

3. Results

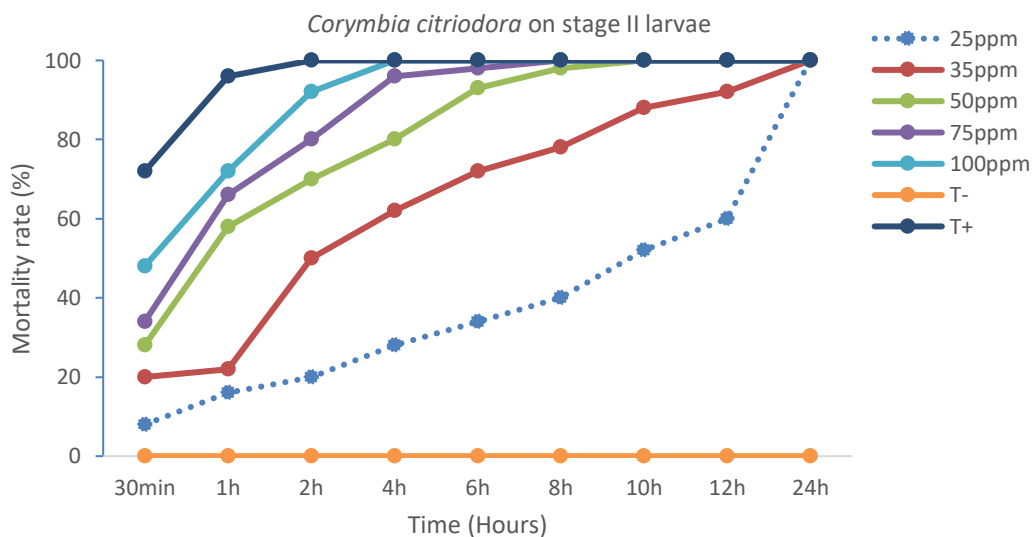
3.1. Larvicidal effect of plant essential oils on *A. gambiae* s.s.

3.1.1. Mortality rate of stage II larvae to essential plant oils

3.1.1.1. Mortality rate of larvae (L2) to *Corymbia citriodora* essential oil

The sensitivity of *Anopheles gambiae* ss stage II larvae to *Corymbia citriodora* essential oil was also observed (Figure I). The lowest concentration (25ppm) began

its larvicidal effect at the 30th minute of exposure, with a mortality rate of 8%, which increased to 100% within 24 hours. The 35ppm, 50ppm, 75ppm and 100ppm doses were more effective, with 100% mortality at the 24th, 10th, 8th and 4th hour of exposure respectively. The chi2 test of homogeneity showed a highly significant difference ($\chi^2= 99.827$; $ddl = 32$, $p\text{-value} = 0.00006759$) between the different mortality rates.

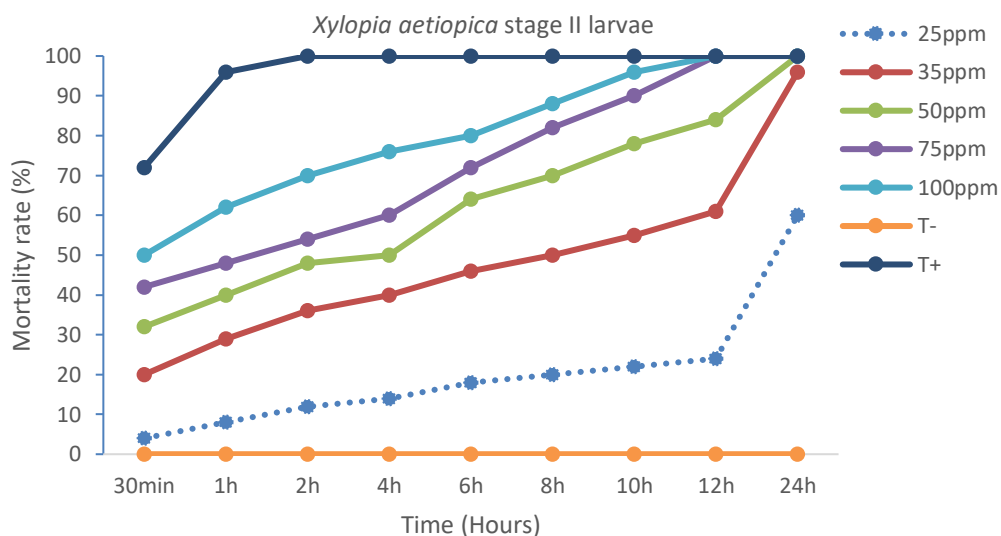


T-: negative control (hexane); T+: positive control (Temephos)

Figure I: Mortality rate of larvae (L2) to *Corymbia citriodora* essential oil

3.1.1.2. Larval mortality rate (L2) with *Xylopi aetiopica* essential oil

All concentrations of *Xylopi aetiopica* test essential oil applied to stage II *Anopheles gambiae* larvae induced mortality from the 30th minute of exposure (Figure II). The lowest concentration (25ppm), which began its larvicidal activity at 30 minutes, inducing 4% mortality, resulted in the death of 60% of *Anopheles* larvae after 24 hours. The highest concentrations (75ppm and 100ppm) resulted in 100% larval mortality after 12 hours' exposure to the *Xylopi aetiopica* essential oil fraction. The other doses, 35ppm and 50ppm, killed 96% and 100% of larvae respectively within 24 hours of exposure. A highly significant difference ($\chi^2= 53.602$; $ddl = 32$, $p\text{-value} = 0.009727$) was observed between these mortality rates.



T-: negative control (hexane); T+: positive control (Temephos)
Figure II: Mortality rate of larvae (L2) to *Xylopiya aetiopica* essential oil

3.1.1.3. LC₅₀ and LH₅₀ of both plants' essential oils on stage II larvae

Lethal concentrations (LC₅₀) and lethal hours (LH₅₀) of *Corymbia citriodora* and *Xylopiya aetiopica* essential oils tested on stage II larvae of *Anopheles gambiae* ss were determined using regression lines. The resulting LC₅₀s and LH₅₀s are shown in Table III.

Table III: Regression equations, LC₅₀ and LH₅₀ of essential oils from the two plants tested on stage II larvae of *Anopheles gambiae* ss

Essentials Oils	Regression Equation	r	LC ₅₀ (ppm)	Regression Equation	r	LH ₅₀ (h/min/sec)
<i>Corymbia citriodora</i>	$y = 2.472x + 1.4586$	0.96	27.07	$y = 1.571x + 4.8993$	0.87	1h 09min 32sec
<i>Xylopiya aetiopica</i>	$y = 2.5731x + 0.8008$	0.96	42.88	$y = 0.9835x + 4.5783$	0.93	2h 41min 02sec

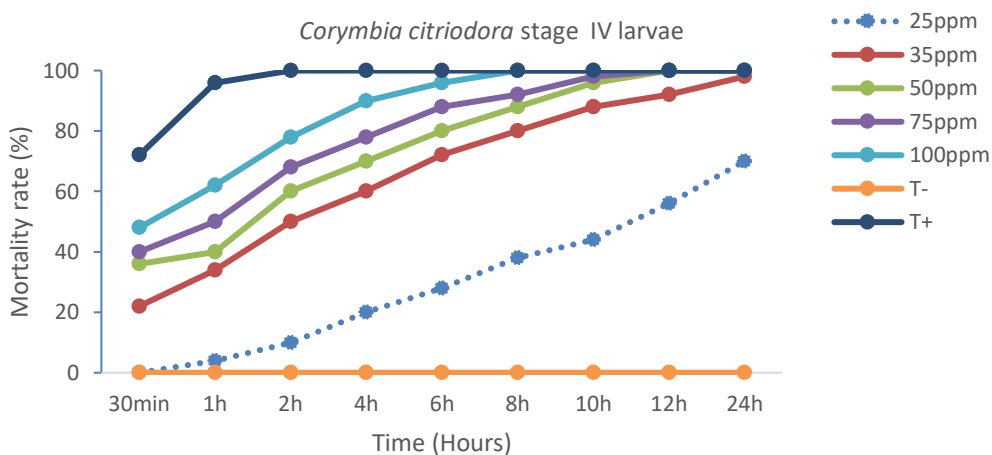
In the light of the data presented in Table III, *Corymbia citriodora* essential oil (LC₅₀= 27.07ppm) has a low LC₅₀ compared with *Xylopiya aetiopica* essential oil (LC₅₀= 42.88ppm). The lethal times determined follow the same order of reactivity, with 1h 09min 32sec for *Corymbia citriodora* essential oil, which revealed faster efficacy than *Xylopiya aetiopica* essential oil (2h 41min 02sec).

3.1.2. Mortality rate of stage IV larvae to essential plant oils

3.1.2.1. Larval mortality rate (L4) with *Corymbia citriodora* essential oil

Variations in the mortality rate of stage IV *Anopheles gambiae* ss larvae exposed to *Corymbia citriodora* essential oil are shown in figure III. This figure shows that the 25ppm and 35ppm doses begin to have a larvicidal effect from the 1st hour and 30th minute, when they induce 4% and 22% mortality respectively, rising to

70% and 98% after 24 hours. Concentrations of 50ppm and 75ppm induce 100% mortality within 24 hours, at the 12th and 8th hour respectively. The highest concentration (100ppm) killed 100% of stage IV *A. gambiae* ss larvae at the 8th hour of exposure. A highly significant difference ($\chi^2 = 82.71$; $ddl = 32$, $p\text{-value} = 0.00002283$) was observed between these mortality rates.

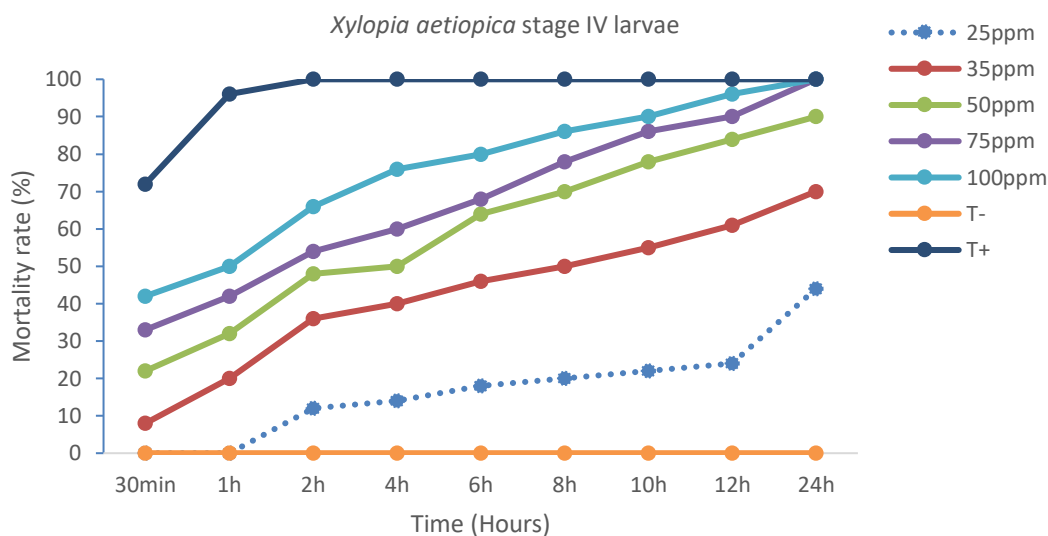


T-: negative control (hexane); T+: positive control (Temephos)

Figure III: Mortality rate of larvae (L4) to *Corymbia citriodora* essential oil

3.1.2.2. Larval mortality rate (L4) with *Xylopiya aetiopica* essential oil

The evolution of mortality rates of *Anopheles gambiae* ss stage IV larvae exposed to different concentrations of *Xylopiya aetiopica* essential oil gave results presented in figure IV. The lowest concentration became active from 2 hours of exposure and induced a mortality rate of 12%, increasing after 24 hours to 44%. Concentrations of 35ppm and 50ppm killed 70% and 90% of *Anopheles* larvae respectively within 24 hours of exposure, while doses of 75ppm and 100ppm eliminated all larvae within 24 hours of exposure. A significant difference ($\chi^2 = 48.124$; $ddl = 32$, $p\text{-value} = 0.03351$) was observed between these mortality rates.



T-: negative control (hexane); T+: positive control (Temephos)
 Figure IV: Mortality rate of larvae (L4) to *Xylopiya aetiopica* essential oil

3.1.2.3. LC₅₀ and LH₅₀ of both plants' essential oils on stage IV larvae

Lethal concentrations (LC₅₀) and lethal hours (LH₅₀) of *Corymbia citriodora* and *Xylopiya aetiopica* essential oils tested on stage IV larvae of *Anopheles gambiae* ss were determined using regression lines. The LC₅₀s and LH₅₀s thus calculated are shown in Table IV.

Table IV: Regression equations, LC₅₀ and LH₅₀ for essential oils from the two plants tested on stage IV larvae of *Anopheles gambiae* ss

Essentials Oils	Regression Equation	r	LC ₅₀ (ppm)	Regression Equation	r	LH ₅₀ (h/min/sec)
<i>Corymbia citriodora</i>	$y = 2.3021x + 1.579$	0.92	30.62	$y = 1.2653x + 4.717$	0,98	1h 40min 25sec
<i>Xylopiya aetiopica</i>	$y = 2.5447x + 0.7251$	0.96	47.85	$y = 0.969x + 4.4519$	0,99	3h 40min 41sec

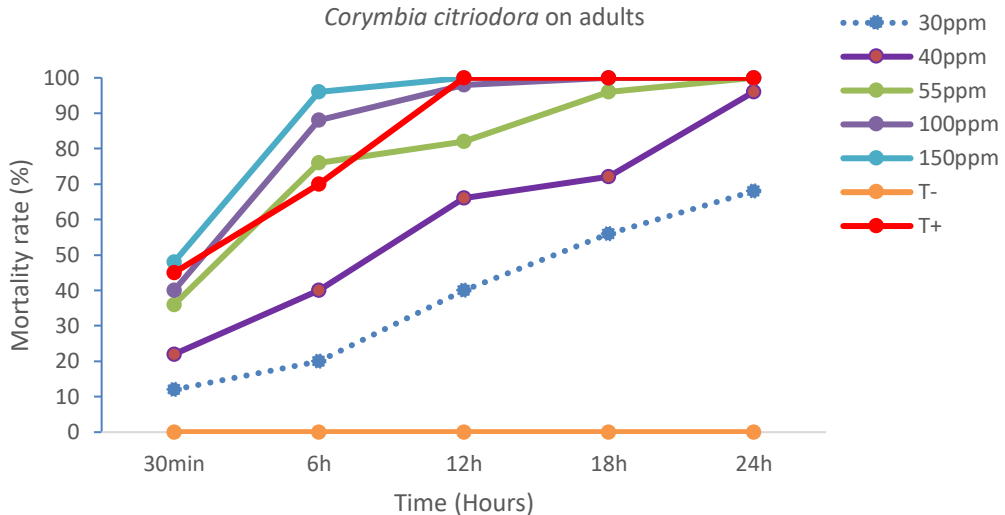
In the light of the data presented in Table IV, *Corymbia citriodora* essential oil (LC₅₀= 30.62ppm) showed low LC₅₀ values compared with *Xylopiya aetiopica* essential oil (LC₅₀= 47.85ppm). The lethal times determined follow the same order of reactivity, with 1h 40min 25sec for *Corymbia citriodora* essential oil, which revealed faster efficacy than *Xylopiya aetiopica* essential oil (3h 40min 41sec).

3.2. Adulticidal effect of plant essential oils on *Anopleles gambiae* s.s.

3.2.1. Adult mortality rate with *Corymbia citriodora* essential oil

The evolution of mortality due to *Corymbia citriodora* essential oil (Figure V) shows that concentrations of 55ppm, 100ppm and 150ppm kill 100% of mosquitoes after 24h. From the 30th minute of exposure, the highest dose (150ppm) induced

mortality 48% higher than the control (45%). At the 6th hour of exposure, concentrations of 55ppm, 100ppm and 150ppm induced more mortality (76%, 88% and 96%) than the positive control (70%).

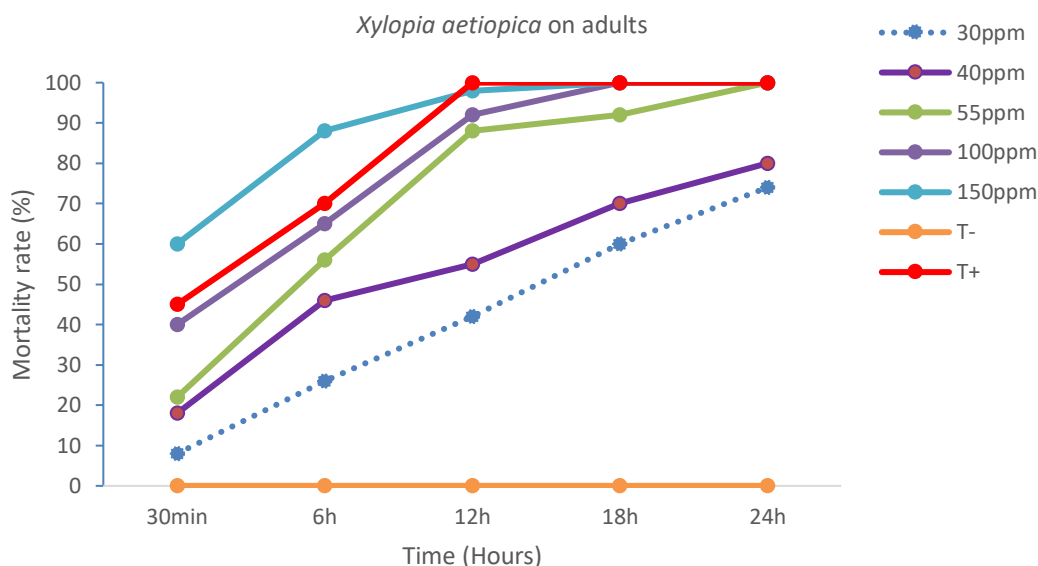


T-: negative control (hexane); T+: positive control (Deltamethrin)

Figure V: Adult mortality rate with *Corymbia citriodora* essential oil

3.2.2. Adult mortality rate with *Xylopiya aetiopica* essential oil

Figure VI below shows the mortality rate of *Anopheles gambiae* ss adults exposed to *Xylopiya aetiopica* essential oil. The figure shows that all concentrations kill 100% of mosquitoes after 24 hours, with the exception of the lowest doses, which induce 74% mortality for 30 ppm and 80% for 40 ppm within 24 hours. From the 30th minute of exposure, concentration 150 induced mortality 60% higher than the control (45%). At the 12th hour of exposure, toxicity becomes low (98%) compared with the control (100%) for the same concentration (150ppm).



T-: negative control (hexane); T+: positive control (Deltamethrin)
Figure VI: Adult mortality rate with *Xylopiya aetiopica* essential oil

3.2.3. LC₅₀ and LH₅₀ of both plants' essential oils on adults

Lethal concentrations (LC₅₀) and lethal hours (LH₅₀) of *Corymbia citriodora* and *Xylopiya aetiopica* essential oils tested on *Anopheles gambiae* ss adults were determined from regression lines. The LC₅₀s and LH₅₀s thus calculated are shown in Table V.

Table V: Regression equations, LC₅₀ and LH₅₀ for essential oils of the two plants tested on *Anopheles gambiae* ss adults

Essentials Oils	Regression Equation	r	CL ₅₀ (ppm)	Regression Equation	r	LH ₅₀ (h/min/sec)
<i>Corymbia citriodora</i>	$y = 2,0039x + 1,977$	0,90	32,25	$y = 1,0437x + 4,7236$	0,84	1h 50min 24sec
<i>Xylopiya aetiopica</i>	$y = 1,9657x + 1,9616$	0,98	35,13	$y = 1,0399x + 4,6447$	0,96	2h 11min 46sec

In the light of the data presented in Table V, *Corymbia citriodora* essential oil (LC₅₀= 32.25ppm) has a low LC₅₀ compared with *Xylopiya aetiopica* essential oil (LC₅₀= 35.13ppm). The lethal times determined follow the same order of reactivity with 1h 50min 24sec for *Corymbia citriodora* essential oil, which revealed a faster efficacy than *Xylopiya aetiopica* essential oil (2h 11min 46sec).

4. Discussion

In *Corymbia citriodora*, high mortality rates were observed in larvae (stage II, then stage IV) compared with the adult stage after 24h of exposure. These results can be explained by the richness of the essential oil in terpenic and phenolic compounds responsible for the observed activity. According to Lahlou (2004), the

biological activity of an essential oil is related to its chemical composition and the possible synergistic effects of its components. Its value lies in the completeness of its constituents, and not just in the majority compounds (Lahlou, 2004). Citriodiol, also known as p-methane-3-8-diol (PMD), is a component of *Corymbia citriodora* essential oil, responsible for its repellent effect (Vera *et al.*, 2014). A study by Isman (2000) on the insecticidal effect of *Corymbia citriodora* essential oil by contact, ingestion and fumigation has been well demonstrated against pests of stored foodstuffs (Isman, 2000). These essential oils act by diffusion, which enables them to reach all the interstices in the stored seed mass, and can therefore be used for fumigation (Koumagalou, 1992). *Corymbia citriodora* essential oil constituents such as hydrocarbon monoterpenes, hydrocarbon sesquiterpenes and sesquiterpene alcohols have shown insecticidal activity (Chao *et al.*, 2005). In our study, *Corymbia citriodora* essential oil showed low LC₅₀ values of 27.07ppm, 30.62ppm and 32.25ppm respectively for stage II larvae, stage IV larvae and adults of *Anopheles gambiae* ss, confirming its very high toxicity. The LH_{50s}, which followed the same order of reactivity (1h 09min 32sec for stage II larvae, 1h 40min 25sec for stage IV larvae and 1h 50min 24sec for *A. gambiae* ss adults), provide ample evidence of the oil's efficacy in terms of time. In view of the above, there is a correlation between LC₅₀ and LH₅₀. Indeed, a plant oil with a low efficacy or a high LC₅₀ will also have a high LH₅₀ (Bouba, 2022). A very positive correlation ($0.84 \leq r \leq 0.98$) was thus noted between LC_{50s} and LH_{50s}. Our results are far superior to those of Njan Nlôga *et al.* (2007) who conducted a study on the efficacy of six essential oils extracted from local plants in North Cameroon on *Anopheles gambiae* sl and found LH_{50s} ranging from 6h 36min 36 sec (*Ocimum canum*) to 101h 23min 24sec (*Pittosporum viridiflorum*) with LC_{50s} of 11.95 mg.m⁻² and 71.79 mg.m⁻² respectively. The difference between our results and those obtained by Njan Nlôga *et al.* (2007) can be explained by the type of plant species used and, above all, the *Anopheles* species tested.

In *Xylopia aethiopica*, the essential oil also showed a low LC₅₀ of the order of 42.88ppm, 47.85ppm, and 35.13ppm respectively for stage II larvae, stage IV larvae and adults of *Anopheles gambiae* ss, confirming its high toxicity. Park *et al.* (2003) have shown that the toxicity of an essential oil varies with the type of plant and also with the species of insect. This variation in toxicity is linked to differences in the chemical composition of essential oils (Regnault, 2002). The efficacy of *X. aethiopica* essential oil is attributable mainly to its high terpene content. Our results corroborate those of Nguemtchouin (2006), who shows that the chromatographic profile of *X. aethiopica* essential oil contains a high number of terpene compounds. In his work, it appears that the contents of sabinene and β-pinene (38.2%), α-pinene (10.2%) and limonene (12.6%) make hydrocarbon monoterpenes the most important group of compounds in *X. aethiopica* essential oil. The metyleugenol content is quite high at 3.6% in the essential oil studied. This essential oil of *X. aethiopica* is therefore very rich in hydrocarbon monoterpenes (MTHC), followed by oxygenated monoterpenes (MTO) such as terpinen-4-ol and sesquiterpenes (ST) (Nguemtchouin, 2006). *Xylopia aethiopica*'s insecticidal properties enable it to combat stock pests (Okonkwo and Okoye, 1996), as well as termites and other wood-boring insects (Lajide *et al.*, 1995). The antimicrobial activity of *X. aethiopica* is also well known (Tatsadjieu *et al.*, 2003). The compounds that give *X. aethiopica* its biological properties include diterpenes such as kauranes, trachylodanes and kolanvanes (Hasan *et al.*, 1982; Harrigan *et*

al., 1994). A study carried out on an oil from Egypt by Karawya *et al.* (1979) shows its particularity with more than two-thirds oxygenated compounds, i.e. 23.4% terpinen-4-ol, 16.3% 1,8-cineole and 11.1% α -terpineol (Noudjou *et al.*, 2004). Koffi *et al.* (2012) in their work on the chemical composition and insecticidal activity of essential oil from the fruits of *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) on *Callosobruchus maculatus* performed GC and GC/MS analysis of *Xylopia aethiopica* essential oil and identified 43 compounds representing over 95% of the essential oil composition: β -pinene (31.92%), germacrene-D (13.04%), α -pinene (10.28%), sabinene (7.88%), and 1,8-cineole (4.87%). This essential oil has an interesting insecticidal activity against *Callosobruchus maculatus*, a cowpea predator in storage, as at the dose of 7 μ L/L of essential oil tested, the mortality rate was over 98% after 24 hours of application (Koffi *et al.*, 2012).

Conclusion

The present work, which aimed to combat malaria by reducing the populations of *Plasmodium* spp. vectors, has shown that the essential oils of *Corymbia citriodora* and *Xylopia aethiopica* possess remarkable larvicidal and adulticidal activities on *Anopheles gambiae* ss. However, *Corymbia citriodora* essential oil was more effective, with LC50s of 27.07ppm, 30.62ppm and 32.25ppm for stage II larvae, stage IV larvae and adults respectively. HL50s followed the same order of reactivity with 1h 09min 32sec, 1h 40min 25sec and 1h 50min 24sec for stage II larvae, stage IV larvae and adults respectively. In view of these results, this plant species could be an intermediate solution in the search for new biocides.

Acknowledgement

The authors of this article would like to express their gratitude to the Organization for the Coordination of the Fight against Endemic Diseases in Central Africa (OCEAC) for supplying the anopheles eggs. They would also like to thank the Head of the Biology Department of the Faculty of Science at the University of Ngaoundere for agreeing to handle the biological tests in the Entomology Laboratory.

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